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Biodegradability Studies with Organic Priority Pollutant Compounds

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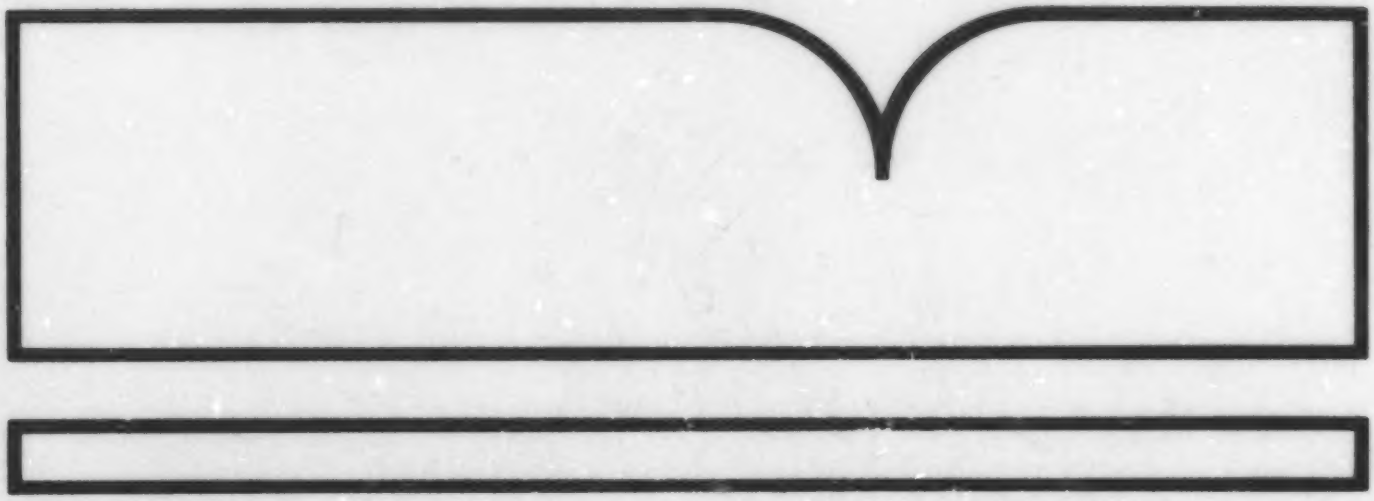

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16. ABSTRACT

Ninty-six organic priority pollutants (from EPA Effluent Guidelines Consent Decree) were studied to determine the extent and rate of microbial degradation and the acclimation periods needed for substrate biooxidation. The pollutants have been classified into groups with characteristic biodegradability patterns.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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Biodegradability studies with organic priority pollutant compounds

Henry H. Tabak, Stephen A. Quave, Charles I. Mashni, Edwin F. Barth

This report deals with studies undertaken to determine the biodegradability of 114 organic priority pollutants included in the U. S. Environmental Protection Agency (EPA) Consent Decree list[1] to ascertain the extent of microbial degradation and to determine the acclimation periods.

With the rapid advances in industrial technology, there is an ever-increasing introduction rate of organic chemicals from the pharmaceutical, petrochemical, oil, solvents, paper, and other industries into water environments. The environmental fate of these organics depends on whether they bio-oxidize rapidly or very slowly, or are biologically recalcitrant, persist in watercourses, become adsorbed to sediment and mud particles and/or bioaccumulate in the food chain process. At the same time, some are toxic to animals and humans, and are physiologically active as well as potentially carcinogenic, posing the possibility of severe health problems.

EPA is consequently very much concerned with ever-increasing introduction of the potentially dangerous pollutants into the watercourses, because of the direct toxic effects on animal and human life or through the reuse of the inadequately treated waste. The agency is charged with the responsibility for setting guidelines and concentration limit standards for the priority pollutants. An intensive effort of determining the biodegradability of these compounds under both laboratory and natural conditions, along with the gathering of the chemical-physical treatability data, is of high agency priority. The acquisition of data on biodegradability and chemical-physical treatability of the priority pollutants is important for the establishment of treatability profiles for the various groups of organic compounds which compose the Consent Decree list.

Data were collected on the degradability and rate of acclimation of 96 compounds.

These biodegradability data are based on studies in which the priority pollutants were subjected to a specific set of controlled experimental conditions and culture-enrichment techniques within the framework of a static culture flask biodegradability screening test.

EXPERIMENTAL PROCEDURE

For this comprehensive screening the priority pollutants were divided into the following classes of organic compounds: phenols, phthalate esters, naphthalenes, monocyclic aromatics, polycyclic aromatics, polychlorinated biphenyls, halogenated ethers, nitrogenous organics, halogenated aliphatics, and organochlorine insecticides.

The biodegradability test method used in the studies was the static-culture flask-screening procedure of Bunch and Chambers,[2] utilizing biochemical oxygen demand (BOD) dilution water containing 5 mg of yeast extract per litre, as the synthetic medium; 5- and 10-mg/l concentrations of the test compound, a 7-day static incubation of 25°C in the dark, followed by three weekly subcultures (totaling 28 days of incubation), and incorporating settled domestic wastewater as microbial inoculum.

The test was modified[3] to include the capability of studying the biodegradation of water insoluble and/or volatile compounds comprising the priority pollutant list and to facilitate the use of both the gas-chromatographic (GC) as well as the dissolved organic carbon (DOC) and total organic carbon (TOC) analytical procedures for determining the extent of biodegradation of the test compound. The procedure was extended to include the experimental initials in the experimental-system series for the original culture and three subcultures to determine the initial concentration of test compound at the beginning of each incubation period. It also incorporated both the medium-inoculum control series to serve as blank controls for determining base lines for both GC analysis and for DOC and TOC, as well as the medium-substrate control series for determination of possible autooxidation, photolysis, and volatilization. Figure 1 shows the incubation of a non-volatile series with cotton-stoppered flasks. Figure 2 shows

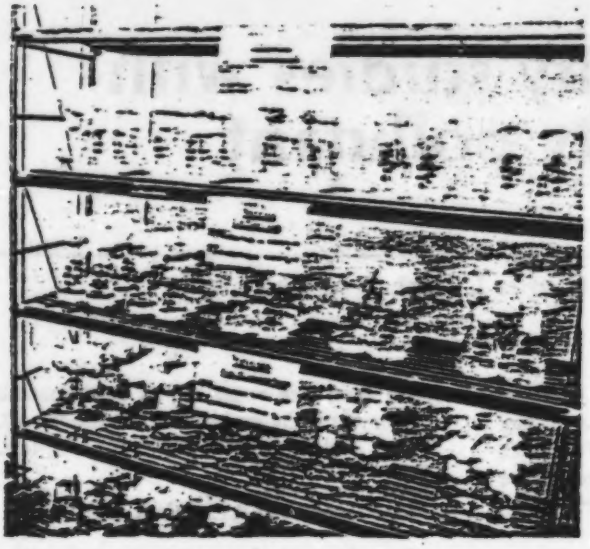

Figure 1—Incubation of non-volatile sample series.

the glass-stoppered vials used during incubation of volatile compounds.

A test protocol was developed to handle the weekly subcultures to fresh media, and to perform solvent extraction of the initial and incubated cultures, evaporate the solvent fractions by the Kuderna-Danish evaporation procedure; cleanup of concentrated solvent extracts for GC analysis and the processing of samples for both the TOC and COD.

Phenol was used as the biodegradable control compound in each biodegradability evaluation series to ensure viability of the wastewater inoculum.

Because each class of organic compounds had different chemical characteristics and solubilities, varied techniques were necessary to provide intimate organism-substrate contact during incubation and unmetabolized substrate recovery.

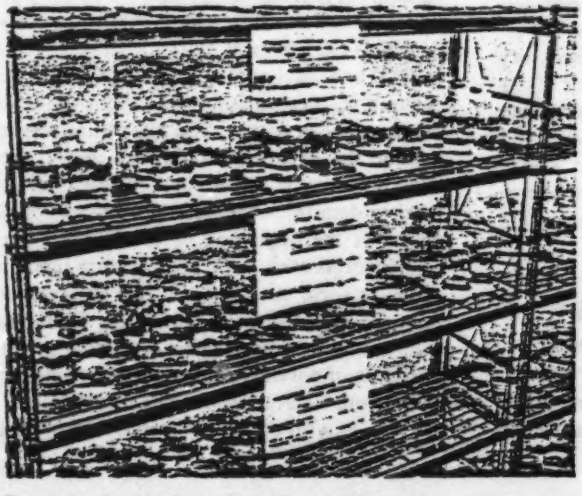

Figure 2—Incubation of volatile sample series.

Phenolic compounds. Stock solutions were prepared in distilled deionized water as 0.1% solutions by addition of sodium hydroxide to effect solution.

The flasks containing the medium, test compound, and inoculum, as well as the medium inoculum and medium test-compound-control flasks, were incubated in a constant-temperature room at 25°C in darkness. Duplicate samples at the beginning of each incubation period and triplicate samples at the end of 7-day incubation for each subculture were subjected to GC and DOC analysis. The culture samples were extracted three times with 20-ml portions of methylene chloride. The pooled-solvent extracts were evaporated by the Kuderna-Danish evaporation technique and the concentrated extracts were then processed for GC analysis. For DOC, the samples were membrane-filtered through a system using 0.22-μm-porosity filters.

The GC methodology was developed for the phenolic compounds present in aqueous culture media and in deionized water as single substrates. The extraction efficiency differed with each phenolic compound and recovery ranged from 87 to 95%. Results were fairly reproducible for several test runs with substrate dosed culture samples. Extractions were performed at pH 2.0.

Phthalate esters and naphthalene compounds. Stock solutions of the test compounds were prepared in absolute ethanol. Solutions of acenaphthene and acenaphthylene were made up as 2 and 4% ethanolic stock solutions respectively, while 10% ethanolic stock solutions were prepared with the remaining test compounds.

Homogenous suspensions of all the phthalate esters, except dimethyl phthalate and diethyl phthalate, and the naphthalene compounds in the synthetic medium were prepared by adding appropriate amounts of the test compound to prechilled synthetic medium in a heavy-duty blender and blending the medium for 2 minutes. Aqueous stock solutions (0.1%) of dimethyl and diethyl phthalates were used in dosing the synthetic medium without blending.

The blended substrate-containing media were then introduced into erlenmyer flasks and inoculated with prechilled yeast extract and settled domestic wastewater inoculum. The flasks containing the medium with the test compound and inoculum, as well as the medium-wastewater and medium-test compound control flasks, were incubated in a constant-temperature room at 25°C in darkness. Flasks were shaken on a daily basis during the incubation period.

The culture samples were extracted three times with 20-ml portions of methylene chloride at neutral pH, except for the control phenol culture samples which were acidified before extraction. The extraction, evap-

oration, and sparging procedure was that used for phenolic compounds.

The extraction efficiency differed with each of the phthalate esters and naphthalene compounds. Recovery ranged from 62 to 149% and was fairly reproducible for the several test runs with substrate-dosed culture samples.

Monocyclic aromatics. Stock solutions of benzene and nitrobenzene were prepared in deionized distilled water. Stock solutions of chlorobenzene, toluene, 2,4- and 2,6-dinitrotoluenes were prepared in absolute ethanol. Both the aqueous and ethanolic stock solutions were prepared as 10% solutions.

A triglyceride (Tributerin®) was used in making up stock solutions of the remaining test compounds, with the exception of hexachlorobenzene, in 10% concentrations. Tributerin® was shown to be a suitable emulsifying agent for the volatile benzenes (chlorinated benzenes and ethyl benzene) and a good vehicle for these extremely water-insoluble compounds.

Homogenous suspensions of ethyl benzene, 1,2-, 1,3-, and 1,4-dichlorobenzenes and 1,2,4-trichlorobenzene in the synthetic medium were prepared by adding appropriate amounts of tributerin® stock solutions of these test compounds to prechilled synthetic medium in a heavy-duty blender and blending the medium for 2 minutes. The same fine suspensions of chlorobenzene, 2,4- and 2,6-dinitrotoluene, and toluene in the synthetic medium were prepared by adding appropriate amounts of the ethanolic stock solutions of these compounds to prechilled synthetic medium. Aqueous stock solutions of benzene and nitrobenzene were used in dosing the synthetic medium without blending.

Hexachlorobenzene, as a 0.1% aqueous suspension, was subjected to ultrasonication for 4 hours. An appropriate volume of the sonicated hexachlorobenzene suspension was then added to prechilled synthetic medium and blended for 2 minutes to make up a fine homogenous suspension of this water-insoluble compound in culture media.

The substrate containing synthetic media were stored in 2-l glass-stoppered reagent bottles in a refrigerator before use.

Biodegradability studies with purgeable benzenes and toluene chlorobenzene, 1,2-, 1,3-, and 1,4-dichlorobenzene, trichlorobenzene, and ethyl benzene were carried out in 250-ml, glass-stoppered reagent bottles to minimize the volatilization of substrate, whereas the nonvolatile benzenes (nitrobenzene, 2,4-, and 2,6-dinitrotoluene, and hexachlorobenzene) were studied in cotton-stoppered Erlenmyer flasks. Both the unblended and blended substrate containing media in either reagent bottles or flasks were inoculated with prechilled yeast extract (5 mg/l) and settled domestic wastewater inoculum. The experimental as well as the medium-wastewater and medium-test compound volatility control bottles and flasks were incubated in a constant temperature room at 25°C in darkness.

Duplicate samples at the beginning of each incubation period and triplicate samples at the end of the 7-day incubation for the original and each subculture were subjected to GC, TOC, and DOC analysis, utilizing the extraction-evaporation methodology, as well as the membrane filtration technology (for DOC determinations) as employed for phenols, phthalates and naphthalenes.

The GC methodology was developed for each of the benzene and toluene compounds, with the emphasis on analyzing single-parent substrate in cultures uncontaminated with the other test compounds. Extraction efficiency differed with each compound and percentage of recovery ranged from 83 to 98% and was fairly reproducible for several test runs with each compound.

Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, halogenated ethers, nitrosamines, phenylhydrazines and herbicides (Isophorone, Acrylonitrile and Acrolein). Stock solutions of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and diphenyl hydrazine were prepared in a polyoxyethylated vegetable oil that was shown to be a suitable emulsifying agent for some volatile chlorinated compounds and a good vehicle for these extremely water-insoluble compounds in aqueous media. Homogenous suspensions of anthracene 1,2-benzanthracene, and chrysene in the synthetic media were prepared by adding appropriate amounts of the solutions of those compounds to the prechilled media and blending it for 2 minutes. The fine suspensions of the other compounds in media were prepared by adding stock solutions to the media without subsequent blending.

Ethanolic stock solutions of haloethers, isophorone, and N-nitroso diphenylamine were used in making fine suspensions of these compounds in prechilled synthetic media without blending. Aqueous stock solutions of N-nitroso-di-n-propylamine, acrylonitrile, and acrolein were used to prepare the culture media.

The substrate containing synthetic media were stored in either 2-l erlenmyer flasks or glass-stoppered reagent bottles in a refrigerator before use.

Biodegradability studies with acrylonitrile, acrolein and three haloethers (4-chlorodiphenyl ether, 2-chloroethyl vinyl ether, and 4-bromodiphenyl ether) were carried out in 250-ml glass-stoppered reagent bottles to minimize volatilization, whereas all the other test compounds were studied in cotton-stoppered Erlenmyer flasks. Both the blended and unblended media in either reagent bottles or flasks were inoculated with prechilled yeast extract and settled domestic wastewater inoculum. The experimental, as well as the medium-wastewater and medium-test compound volatility control bottles and flasks, were incubated in a constant-temperature room at 25°C in darkness. The extraction, evaporation,

and sparging of samples as well as the TOC technology was the same as employed for phenols, phthalates, naphthalenes and benzenes.

The extraction efficiency differed with each of the test compounds and the recovery value ranged from 78 to 98% and were fairly reproducible for several test runs with each of the substrate-dosed cluture samples.

Halogenated aliphatics. Stock solutions of chlorinated ethanes, halomethanes, chloroethylenes, chloropropane and chloropropylene were prepared in absolute ethanol and used for dosing the prechilled synthetic media. Stock solutions were prepared in 10% concentrations. Stock solutions of hexachloro-1,3-butadiene and hexachlorocyclopentadiene were prepared in the polyoxyethylated vegetable oil. Fine suspensions of these two compounds in synthetic media were prepared by adding the appropriate volume of their non-ionic emulsifying agent stock solutions to media without subsequent blending.

The substrate containing synthetic media were stored in either 1- or 2-l glass-stoppered reagent bottles in a refrigerator before use.

Biodegradability studies with the halogenated aliphatics were carried out in 250-ml glass-stoppered reagent bottles to minimize possible volatilization of the test compounds. The substrate containing media in reagent bottles were inoculated with prechilled yeast extract and 10-ml of prechilled settled domestic wastewater inoculum. The experimental, as well as the medium-wastewater control bottles were incubated in a constant temperature room at 25°C in darkness.

Volatility controls, utilizing a nonbiological system (medium-test compound without inoculum) were held at both refrigerated and 25°C holding temperatures for 10 days and then analyzed by GC and for TOC to determine loss of substrate from volatilization.

The experimental cultures, as well as the media and volatility control, were analyzed by a direct injection method (without solvent extraction) chromatographically, with the use of a chromatograph, FID detector system, and a column of SP-1000 on Carbopac B. This procedure applied to chloroethane, halomethane, chloroethylene, chloropropane, and chloropropylene containing cultures.

Butadiene and pentadiene cultures samples were subjected to normal methylene chloride extraction, KD evaporation methodology, and GC analysis.

Organochlorine pesticides. Stock solutions of pesticides were prepared in a non-ionic emulsifying agent. The substrate containing media were stored in either 1- or 2-l glass-stoppered reagent bottles in a refrigerator before use. Appropriate volumes of the oil and stock solutions of pesticides were added to prechilled media and the substrate was thoroughly mixed with the use of a magnetic stirrer. The pesticides were studied in cotton-stoppered erlenmeyer flasks. The substrate containing synthetic media in Erlenmeyer flasks were inoculated with prechilled yeast extract and 10-ml of prechilled settled domestic wastewater inoculum.

The experimental, as well as the medium-wastewater and medium test compound volatility control flasks were incubated in a constant temperature at 25°C. Flasks were shaken for a few minutes on a daily basis during the original and subculture incubation periods.

The extraction of cultures was performed in separatory funnels using three 20-ml volumes of methylene chloride. The KD evaporation of solvent-pooled fractions and the sparging of solvent extracts in preparation for GC analysis was performed in the same manner as phenols, phthalates, benzenes, and naphthalenes.

The extraction efficiency differed with each of the pesticide compounds and recovery values ranged from 75 to 98% and were fairly reproducible for several test runs with each of the substrate-dosed culture samples.

The pesticides were analyzed on a chromatograph equipped with an FID detector system.

RESULTS

The results for each of the above classes are given in the form of a short narrative discussion and tabular presentation of the screening data with correlative data gleaned from the literature.

The indication of 100% biodegradation in the tabular data should not be interpreted as zero residual of the individual priority pollutant. The minimum sensitivity of the GC procedures used was about 0.1 mg/l. The GC analytical effort involved a massive number of analyses in order to verify recoveries, determine blanks, include controls, verify standard chemicals, and monitor replicate samples. In order to keep this effort at a manageable level, the GC methods for each class of compounds were not maximized for sensitivity.

Phenolic compounds. The biodegradability data from the static-culture-flask-biodegradation-screening test studies, based on GC analysis and TOC and DOC determinations, have demonstrated that phenol and the chlorinated, as well as the nitrated, phenols, with the exception of 4,4-dinitro-*o*-cresol, were significantly biodegraded, with either rapid or gradual adaptation periods necessary to achieve optimum biodegradation rates.

The biodegradability data are expressed in Table 1, which summarizes the average percentage of biodegradation for each compound and for each subculture.

Significant biodegradation with rapid acclimation was observed with phenol, 2-chlorophenol, 2,3-dichlorophenol, 2,4,6-trichlorophenol, 2,4-dimethylphenol, *p*-chloro-*m*-cresol, 2-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenol with a range of average biodegradation between 60 to 100% achieved after the first week of incubation. Pentachlorophenol was shown to be signif-

Table 1—Biodegradability of phenolic compounds evaluated by the static-flask-screening method.

Test compound	Concentration of test compound (mg/ml)	Performance summary	Average of 3 test flasks (biodegradation of test compound in 7 days %)			
			Original culture	First subculture	Second subculture	Third subculture
Phenol	5	D	96	100	100	100
	10	D	97	100	100	100
2-Chloro phenol	5	D	86	100	100	100
	10	D	83	100	100	100
2,4-Dichloro phenol	5	D	100	99	98	100
	10	D	99	99	100	99
2,4,6-Trichloro phenol	5	D	100	100	100	100
	10	D	100	100	100	100
Pentachloro phenol	5	A	19	68	100	100
	10	A	16	0	0	99
2,4 Dimethyl phenol	5	D	100	100	100	100
	10	D	99	100	100	100
p-Chloro-*m*-cresol	5	D	78	100	100	100
	10	D	76	100	100	100
2-Nitro phenol	5	D	100	100	100	100
	10	D	100	100	100	100
4-Nitro phenol	5	D	100	100	100	100
	10	D	100	100	100	100
2,4-Dinitro phenol	5	D	60	100	100	100
	10	D	68	100	100	100
4,6-Dinitro-*o*-cresol	5	N	52	58	56	51
	10	N	0	5	11	14

D—Significant degradation, rapid adaptation.
A—Significant degradation, gradual adaptation.
N—Not significantly degraded under the conditions of test method.

icantly bio-oxidized but with a gradual adaptation process over a 2-week period to achieve 100% biodegradation in 5-mg/l substrate cultures and over a 4-week period to achieve 98 to 100% loss in 10-mg/l pentachlorophenol cultures.

The nitrocresol, 4,6-dinitro-*o*-cresol did not demonstrate significant bio-oxidation and necessary acclimation for optimum bio-oxidation activity within the 28-day incubation period under the enrichment-culture conditions of the test method.

In general, chlorophenols are more stable to biodegradation than phenol and the resistance to microbial catabolism is greatest among the more highly chlorinated phenols.[4]

Phthalate esters. Phthalate esters are reported to be metabolized in the aquatic environment by a variety of organisms and degraded by mixed microbial systems at rates which vary widely, depending on environmental conditions. These compounds undergo primary and ultimate biodegradation in naturally occurring microbial populations by mechanisms of enzymic hydrolysis. The rate of degradation depends on temperature, pH, the presence of oxygen and phthalate structure.[5]

The static-culture-flask biodegradability screening test data based on GC and TOC determinations have demonstrated that phthalate ester priority pollutants were significantly biodegraded with either rapid or gradual adaptation to achieve optimum biodegradation rates.

The biodegradability data are expressed in Table 2, which summarizes the average percentage of biodegradation for each test compound and subculture.

Significant biodegradation, with rapid acclimation was observed with the phthalate esters, dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, and butyl benzyl phthalate, with 100% losses achieved after the first week of incubation.

Bis-(2-ethylhexyl) phthalate (EHPE) and di-n-octyl phthalate (DOPE) were significantly bio-oxidized, but with a gradual adaptation process needed over a 3-week period to achieve 95 and 94% loss for EHPE and 94 and 93% loss for DOPE in 5- and 10-mg/l substrate containing cultures respectively at the end of the third subculture incubation. The phthalate esters, EHPE and DOPE, were thus proven the most persistent of the phthalates studies under the conditions of the static-flask screening test.

Naphthalenes. The degradation and metabolism of naphthalene and the identification of the metabolites is known from many studies with bacteria. Naphthalene is reported to be probably the most easily biodegraded polycyclic aromatic hydrocarbon. Biotransformation

Table 2—Biodegradability of phthalate esters and naphthalene compounds evaluated by static-flask-screening method.

Test compound	Concentration of test compound	Performance summary	Average of 3 test flasks (biodegradation of test compound in 7 days (%))			
			Original culture	First subculture	Second subculture	Third subculture
Dimethyl phthalate	5	D	100	100	100	100
	10	D	100	100	100	100
Diethyl phthalate	5	D	100	100	100	100
	10	D	100	100	100	100
Di-n-butyl phthalate	5	D	100	100	100	100
	10	D	100	100	100	100
Bis-(2-ethyl hexyl) phthalate	5	A	0	43	80	95
	10	A	0	47	89	93
Di-n-octyl phthalate	5	A	0	79	89	94
	10	A	0	42	93	92
Butyl benzyl phthalate	5	D	100	100	100	100
	10	D	100	100	100	100
Naphthalene	5	D	100	100	100	100
	10	D	100	100	100	100
2-Chloro naphthalene	5	D	100	100	100	100
	10	D	100	100	100	100
Acenapthene	5	D	95	100	100	100
	10		100	100	100	100
Acenaphthylene	5	D	100	97	96	98
	10	D	94	91	93	93

D—Significant degradation, rapid adaptation.
A—Significant degradation, gradual adaptation.

and biodegradation of naphthalene is rapid enough to make it a dominant fate process in an aquatic system.[6]

The biodegradability data for the priority-pollutant naphthalene compounds based on the static-culture-flask screening test have demonstrated that naphthalene, 2-chloronaphthalene, acenaphthene and acenaphthylene undergo significant biodissimilation in culture media dosed with 5- and 10-mg/l substrate concentration, with rapid acclimation periods for naphthalene oxidizing-enzyme induction to occur. The naphthalene and 2-chloronaphthalene compounds exhibit rapid biodegradation in culture media at the end of the first subculture, showing 100% loss of the substrate at the two concentrations. Acenaphthene was observed to show similarly high biodegradation activity, with 100% loss of substrate in 5- and 10-mg/l dosed cultures throughout the four incubation periods. Rapid dissimilation activity was also demonstrated for acenaphthylene, with ranges of 87 to 100% and 80 to 100% loss of substrate in cultures dosed with 5 and 10 mg/l, respectively, throughout the four 7-day incubation periods.

Table 2 summarizes the biodegradability data for the priority pollutant naphthalene compounds showing the average biodegradation for each test compound for each subculture.

Monocyclic aromatics. Based on the biodegradability data accumulated for the priority-pollutant benzenes, toluenes, and their chlorinated and nitrated derivatives, certain conclusions can be drawn about their relative susceptibility to microbial bio-oxidation, their persistence and stability in aqueous environments as well as about their toxicity to the wastewater microbiota.

The analytical data, based on GC and TOC determinations, have demonstrated significant biodegradation of some of the benzene and toluene compounds, with varying degrees of acclimation depending on the test compound and the dose concentration of substrate in the culture media. Polychlorinated benzenes and the dinitrotoluenes initially exhibited a significant bio-oxidative activity with gradual adaptation and induced enzyme formation, followed, however, by a deadaptive process from the loss of the metabolically efficient microbial population or the gradual build-up of toxicity of either the parent substrate or metabolic products to microbiota. Hexachlorobenzene was shown to be relatively resistant to dissimilation by the microorganisms under the conditions of the static-flask methodology.

Table 3 summarizes the average percentage of biodegradation for each test compound at the end of the original culture and each of the subculture incubation periods. Significant biodegradation with rapid acclimation was observed with benzene, toluene, and nitrobenzene. Toluene and nitrobenzene were shown to be

Table 3—Biodegradability of benzene, toluene and their derivatives evaluated by the static-screening-flask test method.

Test compound	Concentration of test compound (mg/l)	Performance summary	Average of 3 test flasks (biodegradation of test compound in 7 days (%))			
			Original culture	First subculture	Second subculture	Third subculture
Benzene	5	D	49	100	100	100
	10	D	37	95	100	95
Chlorobenzene	5	D	89	100	100	100
	10	A	30	77	100	100
1,2-Dichlorobenzene	5	T	45	66	48	29
	10	T	20	59	32	18
1,3-Dichlorobenzene	5	T	59	69	39	35
	10	T	58	67	31	33
1,4-Dichlorobenzene	5	T	55	61	34	16
	10	T	37	54	29	0
1,2,4-Trichlorobenzene	5	T	54	70	59	24
	10	T	43	54	14	0
Hexachlorobenzene	5	N	56	30	8	5
	10	N	21	3	0	0
Nitrobenzene	5	D	100	100	100	100
	10	D	87	97	100	100
Ethylbenzene	5	D	100	100	100	100
	10	A	89	78	87	100
Toluene	5	D	100	100	100	100
	10	D	100	100	100	100
2,4-Dinitrotoluene	5	T	77	61	50	27
	10	T	50	49	44	23
2,6-Dinitrotoluene	5	T	82	55	47	29
	10	T	57	49	35	13

D—Significant degradation with rapid adaptation.
A—Significant degradation with gradual adaptation.
T—Significant degradation with gradual adaptation followed by a deadaptive process in subsequent subcultures (toxicity).
N—Not significantly degraded under the conditions of the test method.

completely biodegradable after the first week of incubation at 5- and 10-mg/l levels. Benzene demonstrated complete dissimilation at these levels after the second week of incubation. Chlorobenzene and ethyl benzene exhibited complete losses after the second and third weeks of incubation, respectively, at the 5-mg/l level. At 10 mg/l of substrate, biodegradation was manifested by gradual adaptation of microbiota with 100% losses evidenced for chlorobenzene and ethyl benzene at the end of the second and third subculture periods, respectively.

A gradual acclimation process with significant bio-oxidation of the substrate was observed with 1,2-, 1,3-, and 1,4-dichlorobenzene, 1,3,4-trichlorobenzene, and with 2,4- and 2,6-dinitrotoluene. Subsequent subcultures demonstrated a reduction of the biodegradation rate with these test compounds and an accumulation of the parent compound in the culture media.

The gradual reduction of biodegradation activity with respect to the polychlorinated benzenes and dinitrated toluenes may be from the possible loss of synergistic activity on the substrate exhibited by the original heterogenous microbial population in the inoculum as a result of the subculture method, or from a retardation of the adaptive (induced) enzyme process because of possible accumulation of toxic by-products of metabolism.

Hexachlorobenzene did not demonstrate significant bio-oxidation and the necessary acclimation for bio-oxidative activity within the 28-day incubation period under the conditions of the static-flask-culture method.

The volatility control systems for the volatile benzene compounds provided data which demonstrated no significant losses of the substrate from culture media due to volatilization at 25°C holding temperature for 7-day periods.

Some species of soil bacteria and petroleum-degrading microorganisms have been reported to be capable of utilizing benzene, ethyl benzene, and toluene as a sole carbon source and the metabolic pathways involved in the microbial oxidative degradation of these compounds have been established. Nitrobenzene is reported to be potentially biodegradable in soil. The nitro substituent generally decreases the biodegradability of the

Table 4—Biodegradability of polycyclic aromatic hydrocarbons, halogenated ethers and polychlorinated biphenyls evaluated by the static screening flask test method.

Test compound	Concentration of test compound (mg/l)	Performance summary	Average of 3 test flasks (biodegradation of test compound in 7 days (%))			
			Original culture	First subculture	Second subculture	Third subculture
Polycyclic aromatic hydrocarbons						
Anthracene	5	A	43	70	84	92
	10	A	26	30	40	51
Phenanthrene	5	D	100	100	100	100
	10	D	100	100	100	100
Fluorene	5	A	82	53	57	77
	10	A	65	43	38	45
Fluoranthene	5	A	0	47	100	100
	10	N	0	0	0	0
1,2-Benzanthracene	5	N	16	41	35	0
	10	N	0	38	23	0
Pyrene	5	D	71	100	100	100
	10	N	11	2	0	0
Chrysene	5	A	6	65	53	59
	10	N	0	30	34	38
Halogenated ethers						
Bis-(2-chloroethyl) ether	5	D	100	100	100	100
	10	D	100	100	100	100
2-Chloroethyl vinyl ether	5	D	76	75	100	100
	10	D	52	68	100	100
4-Chlorodiphenyl ether	5	N	0	32	36	1
	10	N	0	28	5	0
4-Bromodiphenyl ether	5	N	0	19	36	0
	10	N	0	0	10	0
Bis-(2-chloroethoxy)methane	5	N	0	0	0	0
	10	N	0	0	0	0
Bis-(2-chloroisopropyl) ether	5	D	85	100	100	100
	10	D	83	100	100	100
Polychlorinated biphenyls						
PCB-1016	5	N	44	47	46	48
	10	N	22	46	20	13
PCB-1221	5	D	100	100	100	100
	10	D	99	100	100	100
PCB-1232	5	D	100	100	100	100
	10	D	100	100	100	100
PCB-1242	5	N	37	41	47	66
	10	N	34	33	15	0
PCB-1248	5	N	0	0	0	0
	10	N	0	0	0	0
PCB-1254	5	N	11	42	15	0
	10	N	10	25	0	0
PCB-1260	5	N	0	21	0	0
	10	N	0	6	0	0

D—Significant degradation with rapid adaptation.
A—Significant degradation with gradual adaptation.
N—Not significantly degraded under the conditions of the method.

aromatic ring, and therefore, the polynitrobenzenes are decomposed very slowly. According to reported studies, the more highly halogenated the benzene molecule becomes, the more resistant it is to microbial degradation[7-9].

Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and halogenated ethers.

Polycyclic Aromatic Hydrocarbons. The polycylic aromatic hydrocarbons (PAHS) demonstrated varied rates of biodegradation with different acclimation pe-

riods, depending on the test compound and the dose of substrate in culture media, as shown in Table 4. The tricyclic aromatic hydrocarbon, anthracene, showed significant bio-oxidation with gradual adaptation, with a loss of 92 and 51% of substrate at the end of the third subculture in cultures with initial levels of 5 and 10 mg/l, respectively. Significant biodegradation with gradual adaptation was also demonstrated in the case of fluorene, which exhibited 77 and 45% losses at the end of third subculture with the above initial concentrations.

Fluoranthene, pyrene and chrysene demonstrated significant degradation at 5-mg/l substrate levels, but low oxidative activity at 10 mg/l. The tetracyclic hydrocarbon, 1,2-benzanthracene, in contrast, did not demonstrate significant degradation and the necessary acclimation for oxidative activity during the four successive incubation periods.

The PAHs in general have been reported to be potentially biodegradable compounds, particularly by soil microorganisms and in soil systems which provide better conditions for biodegradation than aquatic systems.[10]

The biodegradability data have shown that the tricyclic aromatic hydrocarbons are more susceptible to biodegradative action than the tetracyclic and higher polycyclic hydrocarbons.

Polychlorinated biphenyls. The individual polychlorinated biphenyls (PCBs) vary widely in their susceptibility to biodegradation, as shown in Table 4. The mono-, di-, and trichlorinated species may be significantly biodegraded or biotransformed, as well as volatilized; whereas PCBs with five or more chlorine atoms per molecule have a tendency to adsorb to suspended materials and sediments, bioaccumulate because of very low solubility, photodissociate, and are quite resistant to biodegradation.[11-15] The priority pollutant PCBs are Aroclors or technical mixtures of individual PCBs made by the partial chlorination of biphenyl in the presence of suitable catalyst. The PCBs 1016, 1221, 1232, 1242, 1254, and 1260 are Arochlors which differ from one another in the average chlorine content on a weight basis and in types of the individual PCBs composing the mixture.

Biodegradability data with the priority pollutant PCB Aroclors, have demonstrated that PCB Aroclor 1221 (with an average chlorine content of 21% and limited to mono- and di-chloro isomers and PCB Aroclor 1232 (with an average chlorine content of 32% and limited to mono-, di-, tri-, and tetra-chloro isomers) were the only PCB Aroclors exhibiting significant biodegradation with rapid acclimation. PCB Aroclor 1016 (a new mixture limited to mono-, di-, tri-, and tetrachloro isomers) and PCB Aroclor 1242 (with an average chlorine content of 42% and limited to di-, tri-, and tetra-chloro isomers) showed little bio-oxidation at 5 mg/l, and no oxidative activity at 10 mg/l. PCB Aroclor 1248 (with an average chlorine content of 48% and limited to tri-, tetra-, and penta-chloro isomers), PCB Aroclor 1260 (with an average chlorine content of 60% and limited to penta-, hexa-, and hepta-chloro isomers) demonstrated recalcitrance to microbial metabolism at 5 and 10 mg/l. The biodegradability of PCBs is a function of the number of C-H bonds available for hydroxylation. The fewer the chlorine atoms, the more common the adjacent unchlorinated carbons, and the higher the rate of bio-oxidative activity.

Halogenated ethers. The 7-day incubation periods for each subculture as shown in Table 4 demonstrated that bis-(2-chloroethyl) ether, 2-chloroethyl vinyl ether, and bis(2-chloroisopropyl) ether are significantly biodegradable with slightly varied acclimation periods for optimum oxidative activity, whereas 4-chlorodiphenyl ether, 4-bromodiphenyl ether, and bis-(2-chloroethyl) methane demonstrated insignificant biodegradability, with successive reduction of bio-oxidative rates and accumulation of the parent compound in the culture media from the possible toxicity of substrate to microbiota.

The findings on the susceptibility of the chloroalkylethers to degradation are corroborated by reported data based on studies with river waters supplemented with wastewater inocula.[16] The haloaryl ethers, as well as the chloroethoxy ethers, are shown to bio-oxidize very slowly under the conditions used. The experimental conditions may not have permitted a proper enzyme induction period or natural selection of microorganisms. It may also be possible that structural configuration of these ethers imparts recalcitrance to dissimilation of the molecule by microorganisms. The static-flask results for these three classes of priority pollutants are shown in Table 4.

Nitrogenous organic compounds. The diphenylnitrosamine, N-nitroso-N-phenylbenzamine (N-nitrosodiphenylamine) was shown to be easily biodegradable by microorganisms, with rapid acclimation to 5-mg/l substrate doses and gradual acclimation to 10 mg/l of the substrate in culture media, whereas the dialkylnitrosamine, di-n-propylnitrosamine (N-nitrosodi-n-propylamine), was observed not to exhibit significant degradation at either concentration.

Based on the relatively few reported data found in the literature, the diphenylnitrosamine is more easily degraded by microorganisms than are dialkylnitrosamines.[17] The carcinogenic nitrosamine, dimethylnitrosamine, which was not included in the biodegradability studies, is reported to exhibit resistance to microbial degradation in soil and in wastewater, and is not affected by the anaerobic organisms in bog sediment.[18]

The hydrazobenzene, 1,2-diphenylhydrazine, exhibited significant biodegradation with gradual adaptation, with the metabolic activity leveling off in the last two

subcultures dosed with 5 mg/l of the substrate. At the initial concentrations of 10 mg/l, the significant bio-oxidative activity exhibited initially with gradual adaptation process, was followed by a deadaptive process and toxification in subsequent subcultures.

The compound is in easily reversible redox equilibrium with azobenzene, and the relative concentrations of the two compounds that will be present at any time depends upon the oxidation potential of the water sample.[19] The azobenzene which predominates in well-aerated water was found in insignificant concentrations in the static-flask medium-substrate control set-ups.

Herbicides. The unsaturated ketone (cyclohexene-one) isophorone was shown to exhibit rapid degradative activity by microorganisms that adapt themselves readily to dissimilate the substrate. The microbial metabolism of isophorone probably involves the oxidation of the allylic methyl group of isophorone to a carboxylic acid group, which is followed by the rupture of the cyclohexane ring and decarboxylation.[20]

The herbicide compound, acrylonitrile (vinyl cyanide) demonstrated ease in degradation with rapid adaptation and induced enzyme formation under the conditions of the enrichment culture technique of the static-flask screening studies. These findings are in agreement with reported studies on acetonitrile biodegradation, which demonstrate that the substrate is readily dissimilated by microorganisms of activated sludge and is not toxic to the microorganisms.[21]

Acrolein (an unsaturated aldehyde) was also shown to be easily dissimilated with rapid acclimation of microbiota to the substrate. These findings are corroborated by reported studies on acrolein biotransformation in activated sludge.[22-24]

Table 5 summarizes the biodegradability data of the studies with the nitrogenous organic compounds.

Halogenated aliphatics. Table 6 summarizes the analytical data based on GC and TOC determinations, and correlates the percentage of losses of the halogenated aliphatic priority pollutants in 7 days for the four successive incubation periods with the average percentage of removals of the substrate from volatilization in 10 days at refrigeration and 25°C.

The volatilization of the halogenated aliphatics reported are based on volatility controls utilizing non-biological systems (synthetic media without inoculum). This approach was used to provide the conditions for the highest possible volatilization in the sealed-bottle systems of the biodegradation screening test to occur, since volatilization is reported to depreciate considerably in solutions containing organic solids and/or microbial cultures.[25]

Chloroethanes. The chloroethane aliphatics, 1,1- and 1,2-dichloroethane, 1,1,1-, and 1,1,2-trichloroethane were shown to be potentially biodegradable, whereas 1,1,2,2-tetrachloroethane was observed not to support any bio-oxidative activity by microbiota under the conditions of the biodegradability test-method used. Tetrachloroethane was shown to exhibit higher resistance to bio-oxidation than the two trichloroethanes (1,1,1-

Table 5—Biodegradability of nitrogenous organics evaluated by the static screening flask test method.

Test compound	Concentration of test compound (mg/l)	Performance summary	Average of 3 test flasks (biodegradation of test compound in 7 days (%)) Original culture	First subculture	Second subculture	Third subculture
Nitrosamines						
N-Nitroso-di-N-propylamine	5	N	27	37	47	50
	10	N	0	8	40	40
N-Nitrosodiphenylamine	5	O	87	100	92	100
	10	A	47	53	95	98
Substituted benzenes						
Isophorone	5	O	100	100	100	100
	10	O	100	100	100	100
1,2-Diphenylhydrazine	5	T	80	73	86	77
	10	T	72	55	40	40
Acrylonitrile	5	O	100	100	100	100
	10	O	100	100	100	100
Acrolein	5	O	100	100	100	100
	10	O	100	100	100	100

O—Significant degradation with rapid adaptation.
A—Significant degradation with gradual adaptation.
T—Significant degradation with gradual adaptation followed by a deadaptive process (toxicity).
N—Not significantly degraded under the conditions of the test method.

and 1,1,2-isomers) which in turn are more refractive to degradation than the two dichloroethanes. Rate of bio-oxidation was also shown to vary depending on the position of the chlorine atom on the C_2 carbon structure. Hexachloroethane, in contrast, exhibits significant degradative activity with rapid adaptation of the microbiota.

These findings are based on considering the relationship of the total percentage of loss data, with the volatility data of the chloroethane aliphatics. Whereas refrigeration temperature did not show any loss of substrate from volatility, losses from volatilization were observed at 25°C for the 10-day holding period. However, considering that the volatility losses were established for non-biological volatility control systems, the volatility losses in experimental biological set-ups were such that the loss of the substrate from biodegradative activity could be inferred. Based on literature data, the chloroethane aliphatics, 1,1- and 1,2-dichloroethane, and 1,1,1-trichloroethane are considered to be potentially biodegradable and will probably be destroyed during the aerobic treatment after a considerable period of acclimation, whereas tetrachloroethanes will exhibit recalcitrance to microbial activity and might be bio-oxidized only after very extensive acclimation periods.[26-28] Because of their lipophylicity, the dichloroethanes should not be bioaccumulated to a large extent, whereas the tri- and tetrachloroethanes, being lipophylic in nature, might have a tendency for accumulation.

Halomethanes. The halomethane aliphatics demonstrate different biodegradation rates and acclimation periods, depending on the test compound and substrate concentration in the culture media. Methylene chloride, bromochloromethane, and carbon tetrachloride were shown to exhibit rapid degradation, whereas chloroform shows significant dissimilation with gradual adaptation. Dichlorobromomethane and bromoform demonstrate 59 and 51% losses and 48 and 35% losses at 5 and 10 mg/l, respectively, at the end of the third subculture period. Chlorodibromomethane and trichlorodifluoromethane exhibit recalcitrance for the four 7-day incubation periods.

As in the case of chloroethane aliphatics, the biodegradability findings for halomethanes are based on evaluation of the effect that volatilization of these compounds might have on the bio-oxidative activity in the microbiota. The total losses of these substrates from culture media, as shown on Table 6, compared to the volatility losses for methylene chloride, bromochloromethane, carbon tetrachloride, chloroform, dichlorobromethane, and bromoform allow biodegradation to be inferred. In contrast, with chlorodibromo- and trichlorofluoromethane, any possible loss of the substrate caused by bio-oxidative action was shown to be precluded by the loss resulting from volatilization.

Chloroethylenes. Based on the analytical data for the four incubation periods of the experimental culture series and on the analysis of the volatility, certain assessments may be made about the biodegradability of the chloroethylene aliphatics. The 1,1-dichloroethylene compound showed relatively significant biodegradation with gradual adaptation at the end of the first subculture period, with leveling off for the next two subculture periods. The volatilization of the substrate in the non-biological volatility control systems at 25°C, although significant, was observed not to be the dominant process, because of the relatively high total precentage of losses of the compound at the end of each successive incubation period.

The 1,2-cis- and 1,2-trans-dichloroethylenes exhibit slow biodegradative activity concomitant with the relatively moderate rate of volatilization established in the non-biological volatility control systems. Based on the total percentage of loss of substrate in culture media and the loss from volatilization, the 1,2-trans-dichloroethylene isomers can be considered more refractive to bio-oxidation than the 1,1-dichloroethylene compound. In general, the dichloroethylene carbon structure seems to confer a significant stability of the molecule to microbial bio-oxidative action.

The chloroethylene aliphatics, trichloroethylene, and tetrachloroethylene, were shown to be significantly biodegradable with gradual adaptation observed. The relatively significant volatilization in the non-biological volatility control series did not preclude the biodegradative activity of microbiota in culture media from the relatively high total percentage of loss of substrate throughout the study in comparison to the volatility percentage losses.

There was no evidence of loss of the substrate from volatilization under refrigeration noticed with the chloroethylenes or the halomethanes.

Dichloropropanes and dichloropropyleses. Biodegradability data indicate that 1,2-dichloropropane and 1,3-dichloropropylene are essentially biodegradable compounds, exhibiting relatively high biodegradative rates, with gradual adaptation of microbiota.

Considering the relatively insignificant volatilization of the chloropropane and chloropropylene aliphatics, the above compounds achieved 89 and 81% losses and 85 and 84% losses at 5 and 10 mg/l, respectively, at the end of the third subculture period. The total percentage of losses at the end of the respective incubation periods in the culture media were essentially from the bio-oxidative activity of the microbiota.

Literature data on 1,2-dichloropropane and 1,3-dichloropropylene corroborate the findings from the static-culture-flask biodegradability screening studies. They are considered biodegradable compounds based on the studies with soil and wastewater microorganisms.[29-31]

Table 6—Biodegradability and volatility studies with halogenated aliphatics evaluated by the static-screening-fla test method.

Test compound	Conc. (mg/l)	Performance summary	Average total loss of test compound in 7 days incubation time (%)				Volatilization loss (%)	
			Original	First	Second	Third	5° C	25°
Chloroethanes								
1,1-Dichloroethane	5	A	50	78	85	91	0	19
	10	A	29	74	79	83	0	4
1,2-Dichloroethane	5	B	26	41	54	63	0	27
	10	B	20	35	51	53	0	5
1,1,1-Trichloroethane	5	B	29	64	76	83	0	27
	10	B	23	53	68	75	0	7
1,1,2-Trichloroethane	5	C	6	30	38	44	0	15
	10	C	0	27	30	39	0	3
1,1,2,2-Tetrachloroethane	5	N	0	12	27	29	0	7
	10	N	0	11	20	23	0	6
Hexachloroethane	5	D	100	100	100	100	0	0
	10	D	100	100	100	100	0	0
Halomethanes								
Methylene chloride	5	D	100	100	100	100	0	25
	10	D	100	100	100	100	0	6
Bromochloromethane	5	D	100	100	100	100	0	11
	10	D	100	100	100	100	0	3
Carbon tetrachloride	5	D	87	100	100	100	0	23
	10	D	80	100	100	100	0	5
Chloroform	5	A	49	85	92	100	0	24
	10	A	46	70	80	100	0	6
Dichlorobromomethane	5	A	35	54	56	59	0	8
	10	A	34	44	46	51	0	1
Bromoform	5	A	11	22	40	48	0	0
	10	A	4	14	31	35	0	0
Chlorodibromomethane	5	N	25	28	35	39	0	16
	10	N	10	12	21	25	0	3
Trichlorofluoromethane	5	N	59	61	65	73	5	58
	10	N	38	40	43	45	2	37
Chloroethylenes								
1,1-Dichloroethylene	5	A	78	100	100	100	0	24
	10	A	45	100	100	100	0	15
1,2-Dichloroethylene-cis	5	B	54	76	79	86	0	34
	10	B	43	75	78	84	0	27
1,2-Dichloroethylene-trans	5	B	67	79	88	95	0	33
	10	B	40	76	81	93	0	26
Trichloroethylene	5	A	64	73	82	87	0	29
	10	A	38	56	76	84	0	22
Tetrachloroethylene	5	A	45	54	69	87	0	23
	10	A	30	41	67	84	0	16
Chloropropanes								
1,2-Dichloropropane	5	A	42	70	79	89	0	3
	10	A	36	61	69	81	0	0
Chloropropylenes								
1,3-Dichloropropylene	5	A	55	65	75	85	0	19
	10	A	54	64	69	84	0	7
Chlorobutadienes								
Hexachloro-1,3-butadiene	5	D	100	100	100	100	0	1
	10	D	100	100	100	100	0	0
Chloropentadienes								
Hexachlorocyclopentadiene	5	D	100	100	100	100	0	0
	10	D	100	100	100	100	0	0

D—Significant degradation with rapid adaptation.
A—Significant degradation with gradual adaptation.
B—Slow to moderate biodegradative activity, concomitant with significant rate of volatilization.
C—Very slow biodegradative activity, with long adaptation period needed.
N—Not significantly degraded under the conditions of test method and/or precluded by extensive rate of volatilization.

Table 7—Biodegradability of organochlorine pesticides evaluated by the static screening flask test method.

Test compound	Concentration of test compound (mg/l)	Performance summary	Average of 3 test flasks biodegradation of test compound in 7 days (%)			
			Original culture	First subculture	Second subculture	Third subculture
Aldrin	5	N	0	0	0	0
	10	N	0	0	0	0
Dieldrin	5	N	0	0	0	0
	10	N	0	0	0	0
Chlordane	5	N	0	0	0	0
	10	N	0	0	0	0
DDT p,p'	5	N	0	0	0	0
	10	N	0	0	0	0
DDE p,p'	5	N	0	0	0	0
	10	N	0	0	0	0
DDD p,p'	5	N	0	0	0	0
	10	N	0	0	0	0
Endosulfan-alpha	5	N	0	0	0	0
	10	N	0	0	0	0
Endosulfan-beta	5	N	0	0	0	0
	10	N	0	0	0	0
Endosulfan sulfate	5	N	0	0	0	0
	10	N	0	0	0	0
Endrin	5	N	0	0	0	0
	10	N	0	0	0	0
Heptachlor	5	N	0	0	0	0
	10	N	0	0	0	0
Heptachlor epoxide	5	N	0	0	0	0
	10	N	0	0	0	0
Hexachlorocyclohexane	5	N	0	0	0	0
α-BHC-alpha	10	N	0	0	0	0
Hexachlorocyclohexane	5	N	0	0	0	0
β-BHC-beta	10	N	0	0	0	0
Hexachlorocyclohexane	5	N	0	0	0	0
ϕ-BHC-delta	10	N	0	0	0	0
Hexachlorocyclohexane	5	N	0	0	0	0
λ-BHC-gamma (lindane)	10	N	0	0	0	0

N—Not significantly degraded or biotransformed under the conditions of the biodegradability test procedure used (phenol control 100 percent degraded).

Chlorobutadienes and chloropentadienes. The compounds, hexachloro-1,3-butadiene and hexachlorocyclopentadiene, demonstrated a 100% loss of substrate at 5 and 10 mg/l at the end of the original culture incubation period and this bio-oxidative activity continued over the next three 7-day incubation periods. The volatility control systems showed almost no loss from volatilization of the two compounds at both the refrigeration and 25°C holding temperatures.

These data are in agreement with reported studies on hexachlorocyclopentadiene, which was shown to be degradable by the microorganisms of wastewater sludge and of an aquatic ecosystem.[32,33]

Organochlorine insecticides. The priority pollutants consisting of aldrin, dieldrin, chlordane, DDT p,p', DDE p,p', DDD p,p', endosulfan-alpha, endosulfan-beta, endosulfan sulfate, heptachlor, heptachlor epoxide, endrin, hexachlorocyclohexane isomers (alpha, beta, and delta) and lindane compounds were shown to be recalcitrant to bio-oxidative activity of wastewater microbiota at 5 and 10 mg/l for the four successive 7-day incubation periods of the static-flask-culture method. Data based on chromatographic analysis of methylene chloride extracts indicate 0% losses of the above insecticide compounds. The chromatographic results indicate no reduction of the chromatographic peaks characterizing each parent pesticide and concomitant absence of new peaks with different retention times suggesting possible transformation metabolites, thus providing evidence of persistence of the parent substrate.

Table 7 summarizes the biodegradability data with organochlorine insecticides for the successive 7-day incubation and subculture periods. Evidence of absence of any biodegradative activity in the static culture flask studies is corroborated by the results from literature-reported studies, with regards to the organochlorine pesticides, dieldrin, chlordane, DDE, DDD, endosulfan sulfate, endrin, and heptachlor epoxide.

Possible transformation activities by microorganisms in the static-culture-flask studies, such as epoxidation of aldrin to dieldrin; cometabolic transformation of DDT to DDE; possible transformation of endosulfan isomers to endosulfan sulfate and heptachlor to heptachlor epoxide; and transformation reaction of alpha, beta, and delta BHC isomers, as well as of lindane to metabolites, if present, were not established by the analytical procedures used.

These results on the chlorinated insecticides are consistent with published work on this class of compounds which show resistance to biodegradation.[34-36]

Priority pollutants not evaluated by the static-flask test. This current work with priority pollutants comprises a total of 96 organic compounds belonging to several well-defined chemical classes. The EPA Consent Decree list includes 114 organic compounds. The additional 18 compounds were not included in the static culture flask biodegradability screening studies for the following reasons:

Polycyclic Aromatic Hydrocarbons		
Benzo(a)-pyrene	Carcinogenic compound	Included in Illinois Institute of Technology (IIT) Research Institute Contract Studies, Chicago, Ill. (EPA Contract #68-03-2834)
3,4-Benzofluoranthene	Carcinogenic compound	
Benzo(k)fluoranthene	Carcinogenic compound	
Benzo(g,h)perylene	Carcinogenic compound	
Dibenzo(a,h)anthracene	Carcinogenic compound	
Indeno(1,2,3-cd)pyrene	Carcinogenic compound	
Pesticides		
Toxaphene	Toxic or carcinogenic compound	Included in ITT Research Institute Contract Studies, Chicago, Ill.
Dioxin	Toxic or carcinogenic compound	
Endrin aldehyde	Compound unavailable	
Arylamines (biphenylamines)		
Benzidine	Carcinogenic compound	Included in IIT Research Institute Contract Studies Chicago, Ill.
3,3'-Dichlorobenzidine	Carcinogenic compound	Published Continuous Feed Reactor Studies[37]
Haloethers		
Bis-(2-chloromethyl) ether	Toxic and/or carcinogenic compound	Included in IIT Research Institute Contract Studies, Chicago, Ill.
N-nitrosodimethylamine	Carcinogenic compound	Included in IIT Research Institute Contract Studies Chicago, Ill.
Chloroethanes and Halomethanes		
Vinyl chloride	Carcinogenic compound	Included in IIT Research Contract Studies Chicago, Ill.
Chloroethane	Gas at room temperature	
Methyl chloride	Gas at room temperature	
Methyl bromide	Gas at room temperature	
Dichlorodifluoromethane	Gas at room temperature	

DISCUSSION

The static-culture-flask biodegradation screening technology used in this study made it possible to determine the biodegradability of each of the priority pollutants at two concentrations (5 and 10 mg/l) by wastewater microbiota, utilizing culture-enrichment techniques. Under normal waste treatment conditions, such culture-enrichment processes are different and the feed and sludge retention times may not always be optimum for the establishment of an acclimated microbial population to efficiently treat the priority pollutant. Furthermore, the concentration levels of the organic pollutants in wastewater will be significantly smaller than those used in the biodegradation studies, and as such, may not elicit an induction enzyme formation response by the activated sludge microbiota.

The assessments of the biodegradability of the priority pollutants with the use of the static-culture-flask method can be helpful, however, in predicting the treatability of these compounds by the normal wastewater treatment process, provided methodology is modified to allow for adaptation to these compounds to occur. The static-culture-flask data can be utilized to predict the possible recalcitrance, stability, and persistence in the effluents and in natural bodies of water, following effluent discharge, as well as the potential toxicity of the parent compound or their metabolites to microbiota of activated sludge during the waste treatment.

The completed studies have established a biodegradability profile for the priority pollutants constituting several classes of organic compounds included in the Consent Decree list. The findings from the biodegradability studies could be utilized in the development of bio-oxidative predictability patterns for closely related compounds.

In some cases, it was possible to compare the results of the simple static screening tests with published work that utilized a variety of biodegradation procedures. In all such cases, good agreement was noted.

The priority pollutants that were observed not to exhibit significant degradation under the conditions of the static-culture-flask methodology, cannot be presumed to be completely recalcitrant to microbial action. More rigorous biodegradability methods, such as shaker-flask techniques, soil percolation studies, aerated batch fermentation set-ups, and the continuous-feed aerated reactor technology could be utilized to provide optimum conditions for acclimation of microorganisms to dissimilate the compounds, and thus definitely ascertain the biodegradability of the pollutant that did not pass the static-culture-flask screening test.

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Authors. Henry H. Tabak, Stephen A. Quave, Charles I. Mashni, and Edwin F. Barth are all research chemists with the Municipal Environmental Research Laboratory of EPA, Cincinnati, Ohio.

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